CHAPTER I

INTRODUCTION

Fossil fuels reserves are rapidly diminishing. The fuel sources to replace fossil fuels must be renewable, sustainable, efficient, cost-effective, convenient and safe (Najafpour et al., 2004). Therefore, ethanol production has grown dramatically in the last few years as clean renewable fuel. However, in Thailand, ethanol production by yeast fermentation in hot climates has generally been found uneconomic without government subsidy. This is because of the high energy input required to maintain the fermentation process temperature between 25°C and 35°C to maximize ethanol production (Banat et al., 1992).

Ethanol fermentation at high temperature has received much attention for effective ethanol production in topical counties where average day-time temperatures are usually high throughout the year (Limtong et al., 2007). The advantages associated with the production of ethanol at high temperature include increased rate of productivity, reduced cooling cost, reduced risk of contamination and suitability for use in tropical countries. The well-known yeast producers, *Saccharomyces cerevisiae* and *Zymomonas mobilis* are candidates for ethanol production. *Saccharomyces cerevisiae* has an advantage for its tolerant to high sugar and ethanol concentration. It has previously been demonstrated that *Saccharomyces cerevisiae* M30 is capable of producing ethanol at temperature ranging from 30 to 35°C when grows on media containing cane molasses (Phisalaphong et al., 2006). Recently it has been demonstrated that the thermotolerant yeast strain *Kluyveromyces marxianus* DMKU 3-1042 was an effective strain that could be employed for ethanol production at elevated temperature up to 45°C when sugar cane juice was used as a raw material (Limtong et al., 2007). In order to improve the ethanol fermentation process, the use of a mixed culture of these strains, *S. cerevisiae* M30 and *K. marxianus* DMKU 3-1042 is applied in this study. The batch fermentation by the mixed suspension culture is evaluated using shake flasks. Furthermore, the immobilization of the mixed culture

is evaluated in batch fermentation using shake flasks and in continuous fermentation using a packed bed column.

1.1 Objectives

1.1.1 To evaluate ethanol fermentation by the mixed cultures of *Saccharomyces cerevisiae* M30 and *Kluyveromyces marxianus* DMKU 3-1042 in form of suspension culture and immobilized culture.

1.1.2 To develop continuous ethanol fermentation process using the immobilized mixed-culture of *Saccharomyces cerevisiae* M30 and *Kluyveromyces marxianus* DMKU 3-1042 in a packed bed reactor.

1.2 Expected benefits

1.2.1 Invention of high performance ethanol fermentation process that can work in a wide temperature range.

1.2.2 Useful information for a better understanding of the mixed culture system

1.3 Working scopes

In this work, the ethanol fermentation using the mixed culture system was evaluated in form of the suspension culture and immobilized culture. The working scopes are as follows:

1.3.1 Flocculating yeasts strains, *Saccharomyces cerevisiae* M30 and *Kluyveromyces marxianus* DMKU 3-1042 are used as ethanol producers.

1.3.2 The study of suspension culture and the preliminary study of immobilized culture are carried out in batch mode in a temperature range of 33 - 45°C.

1.3.3 Thin shall silk cocoons and loofa sponges doped with alginate are used for the immobilization in 1.3.2.

1.3.4 Sugar cane juice and cane molasses are utilized as carbon and energy source in 1.3.2.

1.3.5 Batch fermentation is carried out in shake flask culture system at shaking frequency of 200 rpm and at initial sugar concentration of 220 g/l with the initial pH of 5.0.

1.3.6 The suitable method for cell immobilization from the study of 1.3.2 is used for the experimental study in a continuous mode.

1.3.7 The continuous fermentation is carried out in a packed-bed reactor with the working volume 0.7 liters (6 cm diameter and 34 cm height) with the dilution rate varied from 0.1, 0.2, 0.3 and 0.4 h^{-1}.